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Description

[Cosmeceutical and nutraceutical applications of Garcinia extract containing minimum of 3% polyisoprenylated compounds for skin, hair and nails]

BACKGROUND OF INVENTION

[FIELD OF INVENTION]

[0001] This invention discloses new cosmetic applications of lipophilic Garcinia fruit extracts containing a minimum of 5% polyisoprenylated benzophenones particularly 3% Garcinol and 2% cambogin. The fruit extracts are useful as specific metalloprotease inhibitors (against the enzymes elastase, collagenase) and the enzyme hyaluronidase, with potential applications in alleviating signs of premature aging, photoaging, fine lines, crows feet, wrinkles, and in strengthening elasticity, tone, and texture of the skin and

hair. These benzophenones are also useful as antibacterial agents in the treatment and prevention of acne. Additionally these benzophenones are useful in inflammatory conditions as cyclooxygenase inhibitors. The invention also discloses a simple one step, solvent free process for commercial manufacture of cambogin from garcinol.

[Description of Prior Art]

[0002] Indian Patent 160753 reported the process for extraction of garcinol, hydroxycitric acid and anthocyanins for application in food industry as coloring additives from the plant Kokum (Garcinia indica).

[0003] US Patent 5,972,357 reported invention related to healthy food and cosmetics containing derivatives of polyisoprenylated benzophenones from Garcinia as effective ingredients for anti-ulcer activity, Maillard reaction inhibition, antioxidation reactive species scavenging activity and anti-tumor promotion activity.

[0004] Yet another patent (JP 2000-044468) describes the lipase inhibition and its use as anti-obesity medicine and hyperlipidemia inhibitor.

[0005] JP 2000-072665 describes polyisoprenylated benzophenones as hyaluronidase inhibitor with at a daily dose of 10 mg to 10g per adult.

[0006] JP 8259493A2 discloses the use of garcinol from Garcinia suberiptica, as antibacterial activity against methicillin-resistant Staphylococcus aureus (MRSA) effective for suppressing the development of resistant strain, capable of decreasing the amount of antibiotic substance needed, when combined with an antibiotic substance.

[0007] JP 1113965A2 discloses the use of medicine containing benzophenone derivatives for carcinogenicity preventing effects having induction suppressing activity of an Epstein-Barr virus early antigen (EBV-EV).

[0008] JP 10121044A2 discloses the antioxidant, active hydrogen eliminating properties of benzophenones from Garcinia for preventing oxidation of fat and oils and preventing various disturbances of a living body.

[0009] Yamaguchi et al (J. Agric. Food Chem. 2000, 48(6) 2320-5) describe the use of Garcinol from Garcinia fruit for preventing free radical scavenging activity and preventing acute ulceration induced by indomethacin and stress.

[0010] JP 11029465A2 reported the use of Garcinol and derivatives as anti-tumor agent useful for preventing and treating tumor of digestive system.

[0011] Bakana P et al (J. Ethnopharmacology 1987, 21(1) 75-84)

reported the chemotherapeutic activity of polyisoprenylated benzophenones from Garcinia huillensis against Gram positive , gram- negative cocci, mycobacteria and fungi.

[0012] US patent 20020187943 discloses bioavailable composition of natural and synthetic hydroxycitric acid (HCA) in combination with either one or both of Garcinol and anthocyanin, and its use as a weight-loss therapy in animal subjects, preferably humans. The antioxidant properties of Garcinol are described as being enhanced in the presence of HCA and anthocyanin, and the combination of HCA, Garcinol and anthocyanin is also shown to exert greater citrate lyase inhibiting properties than either compound alone. Methods of obtaining HCA, Garcinol or anthocyanin, or a composition containing all three compounds, are described.

[0013] Surprisingly, none of the prior art described above disclose the anti-bacterial activity against anaerobic microorganism Propionibacterium acnes, the acne and pimple causing bacteria, nor do they disclose the metalloprotease inhibition activity especially the elastase and collagenase, broad spectrum UV protection property due to its longer critical wavelength nor its use in preventing hyper-

proliferative skin and nail diseases like psoriasis, eczema, seborrhic dermatitis among others, for providing topical or oral benefits.

[0014] *References*:

[0015] Indian Patent 160753 , A process for the extraction of Garcinol hydroxycitric acid and anthocyanins which are useful in food industry as coloring additives from the plant Kokum (Garcinia indica) Council of Scientific and Industrial Research, 1985

[0016] Yamaguchi et al Healthy foods and cosmetics US Patent 5,972,357, 1999

[0017] Yamaguchi et al., Lipase inhibitor and antiobestic medicine or hyperlipidemia inhibitor. JP 2000-044468 2000

[0018] Yamaguchi et al., Hyaluronidase inhibitor. JP 2000-072665

[0019] Yamaguchi et al., Free radical scavenging and antiulcer activity of Garcinol from garcinia indica fruit rind. J. Agric. Food Chem. 2000, 48(6) 2320-5

[0020] Saito et al., Antitumor agent. JP 11029465A2, 1997

[0021] Iinuma Munekazu., Anti-MRSA active substance JP 8259493A2, 1995

[0022] Yamaguchi et al., Antitumor agent. JP 1113965A2, 1999

[0023] Yamaguchi et al., Antioxidant, active hydrogen eliminator and their application. JP 10121044A2, 1998

[0024] Bakana P et al., J. Ethanopharmacology 1987, 21(1) 75-84

[0025] Majeed et al., Bioavailable composition of natural and synthetic HCA. US patent 20020187943, 2002

SUMMARY OF INVENTION

[0026] One of the main objectives of this invention is to identify natural antibacterials especially against Propionibacterium acnes, the major causative anaerobic organism in acne and pimples. This organism thrives on triglycerides of sebum, by releasing lipid hydrolyzing enzyme lipase, breaking down longer fatty acid triglycerides to smaller chain acids like propionic acid among others. The smaller chain carboxylic acids trigger inflammatory responses causing pustules in severe acne conditions. Another objective of this invention is to identify natural metalloprotease inhibitors particularly against elastase, collagenase, and the enzyme, hyaluronidase, which can be safely used in cosmetic compositions for arresting the signs of aging such as loss of elasticity, tone, firmness, suppleness.

[0027] Another objective of this invention is to identify cyclooxy-

genase inhibitors. Cyclooxygenases are a class of bifunctional enzymes, which catalyze the synthesis of prostaglandins and thromboxanes from arachidonic acid in the body. Prostaglandins regulate many physiological processes including platelet aggregation, uterine contraction, pain, inflammation and secretion of mucins from gastric mucosa. Thromboxanes induce vasoconstriction and platelet aggregation, early steps in blood clotting. Cyclooxygenase inhibitors especially from natural products could find potential application topically or orally, as analgesic, anti-inflammatory, reduce the probability of heart attacks, strokes, aches, sore muscles, fever and hyperproliferation conditions.

[0028] Another objective of the invention is to identify stable natural actives which has longer critical wavelength, that can be used in topical preparations as UVA and UVB protectant.

[0029] Another objective of the invention is to identify natural actives which can control increase proliferation of cells, particularly epethelial cell that can be used topically or orally to treat or prevent hyperproliferative conditions like psoriasis, keloid, dandruff, eczema, carcinoma, among others. Yet another objective of the invention is to develop

a commercially viable process of manufacture of Cambogin which we have developed using a single step, process from Garcinol.

[0030] By use of Garcinia extract containing a minimum of 3% Garcinol and 2% Cambogin benzophenones, which are the lipophilic components of Garcinia, we have met the above mentioned objectives.

Detailed Description

[0031] Garcinol and cambogin belong to a distinct class of compounds calle polyisoprenylated benzophenones. These are abundantly found in various species of Garcinia fruit including Garcinia cambogia, Garcinia indica, Garcinia huillensis among others. Prior art teaches the isolation of benzophenones from Garcinia fruit, and the use of benzophenones particularly Garcinol and Isogarcinol as antiobesity, hypolipidemic and as antitumor agents for the digestive system. We have now surprisingly discovered that Garcinol, cambogin and their related benzophenone derivatives fronm Garcinia fruit are powerful antibacterial agents against Proipionibacterium acnes. These compounds also inhibit elastase, collagenase, cyclooxygenase enzymes, offer broad spectrum UV protective action and are a powerful anti-proliferative agent also inhibit elas-

tase, collagenase, cyclooxygenase enzymes, offer broad spectrum UV protective action, and are a powerful anti-proliferative agent that can be used topically in cosmetic and pharmaceutical applications.

[0032] The findings and observations are described by the following examples.

[0033] *Example 1 Antimicrobial study against Propionibacterium acnes* To carry out the antibacterial activity of the products against P. acnes, the organism was first cultured in an anaerobic chamber (from Coy Laboratory, USA, with an automatic air lock purge system). To standardize the growth of anaerobic culture of P. acnes in the anaerobic chamber, different culture media were used. The anaerobic condition in the chamber was maintained by initialization with Nitrogen gas and then the mixture of gases of Nitrogen and hydrogen and carbon dioxide (80:10:10). A two gas tank arrangement was made wherein only Nitrogen was connected to the transfer chamber and mixed gas to the main chamber using Gassing Manifold (Hrishi Biotech. Pune, India). The anaerobic media were prepared by heating the media while passing the mixture of gases of Nitrogen and Carbon dioxide in 4 :1 proportion simultaneously. The media were added with a redox indicator (resazurin:

0.001%) which is colorless in the presence of anaerobiasis and shows blue color in its absence. It was dispensed to 30ml, 20ml, 10 ml vials sealed with rubber and aluminum clamps and sterilized by autoclaving at 121 deg. C for 15 minutes.

[0034] The culture used in the study is Propionibacterium acnes ATCC 11827. The culture from the broth was inoculated to the fresh medium in vials (10%) and incubated for 48 hours at 37 deg C. The optical density (OD) of the culture at 625 nm was measured. It was maintained between 0.64 and 0.80 which corresponds to approximately 12 x 10^6 cells/ml (4.0 MC Farland standard). Medium used for this study was reinforced clostridial agar (RCA). The medium that was prepared and sterilized in 30 ml vials was poured into the plates inside the chamber and allowed to solidify. The culture was inoculated (0.3 ml per plate) into the plates and spread. After 30 minutes, antibacterial sterile discs (6 mm) were dispensed (2 per plate). 2.5, 5.0, 7.5 and 10 µL of the prepared samples and controls were dispensed onto the discs. The plates were incubated inside the anaerobic chamber at 37 deg C for 48 hours duration. Garcinol and Cambogin in different concentrations (0.1to 2%) prepared in DMSO and was used as vehicle for the

study with DMSO as control. Clindac A (Clindamycin Phosphate Gel 1% w/w) was used as positive control. The clearance zone formed around the discs were measured and expressed in mm.

[0035] The results of the study are shown in Table 1:

Table: 1

Sl. No.	Conc. of the sample (%)	Zone of Inhibition (in mm) Extract A	Extract B	Extract C	Garcinol 90%	Combogin 90%	Clindac – A
1	5.0	7	8	10	20	0	20
2	2.0	0	7	8	19	0	16
3	1.0	0	0	8	18	0	7
4	0.5	0	0	7	16	0	0
5	0.3	-	-	0	14	-	0
6	0.1	-	-	0	11	-	0
7	0.05	-	-	-	10	-	0
8	0.03	-	-	-	8	-	0
9	0.01	-	-	-	7	-	0

Extract A = Garcinia extract containing 3% Garcinol and 2% Cambogin Extract B = Garcinia extract containing 20%Garcinol and 10% Cambogin Extract C = Garcinia extract containing 40% Garcinol and 20% Cambogin

[0036] As seen in the results, the effect of the extract is dependent on the content of Garcinol. Higher the purity of Garcinol from Garcinia fruits, better is the activity against P. acnes is excitingly showing very good inhibition of Propionibacterium acnes. This is showing the inhibitory activity at the minimum concentration of 0.01% of 90% Garcinol (0.009%) is as potent as Clindac-A at 1 % concentration of 1% preparation (0.01%) .On the other hand, Cambogin, a

closely related benzophenone did not show any inhibition of P.acnes even at the concentration of 25%. This clearly indicates that presence of free hydroxyl group at position 7 of Garcinol is essential for the activity of Garcinol.

[0037] *Example 2 Collagenase inhibitory activity of Garcinia benzophenones* The assays were done using the EnzChek collagenase assay kit. The substrate was DQ gelatin (from pig skin). The reduction in fluorescence intensity was measured in a microplate reader Fluostar Optima (emission at 485nm and excitation at 520nm). Aliquots of the enzyme solution (100µl of 0.4U/ml collagenase Type IV from Clostridium histolyticum) and different concentrations of the material in DMSO (80µl) were preincubated in a microplate for 10 minutes. After preincubation, 20µl of the substrate DQ gelatin (12.5µg/ml) was added and the fluorescence intensity was measured after 30 minutes. Enzyme activity with DMSO and controls were also taken. The final concentration of DMSO in the reaction mixture was 3%, which did not show any significant effect on the enzyme activity.

[0038] The percentage inhibition is calculated as follows:

$$\% \text{ Inhibition} = \frac{(B-C) - (T-C)}{(B-C)} \times 100$$

B = Fluorescence in the presence of enzyme
C = Fluorescence of the inhibitor alone
T = Fluorescence of the enzyme activity in the presence of inhibitor

[0039] The results are shown in Table 2.

Table 2 : Effect of Garcinia extract containing different concentrations of Garcinol a Cambogin on collagenase activity

Garcinia Extracts (Percentages of Garcinol and Cambogin)	IC 50
Extract (3% Garcinol + 2% Cambogin)	191 mcg
Extract (20%Garcinol + 10% Cambogin)	108mcg
Extract (40% Garcinol + 20%Cambogin)	48 mcg
Garcinol 90%	24 mcg
Cambogin 90%	6 mcg

Conclusion : Garcinol is 4 times as potent as cambogin

[0040] *Example 3: Elastase inhibitory activity of Benzophenones from Garcinia* The assays were done using the EnzChek elastase assay kit.The substrate is DQ elastin-soluble bovine neck ligament elastin labeled with BODIPY FL dye such that the conjugate's fluorescence is quenched. The reduction in fluorescence intensity was measured in a mi-croplatereader/ Fluostar Optima (emission at 485nm and excitation at 520nm). Aliquots of the enzyme solution (100µl of 0.5U/ml of elastase from pig pancreas) and dif-

ferent concentrations of the material in DMSO (50µl) were preincubated in a microplate for 10 minutes. After preincubation, 50µl of the substrate DQ elastin (25µg/ml) was added and the fluorescence intensity was measured after 30 minutes. Enzyme activity with DMSO and controls were also taken. The final concentration of DMSO in the reaction mixture was 3%, which did not show any significant effect on the enzyme activity.

[0041] The percentage inhibition is calculated as follows:

$$\% \text{ Inhibition} = \frac{(B-C) - (T-C)}{(B-C)} \times 100$$

B = Fluorescence in the presence of enzyme
C = Fluorescence of the inhibitor alone
T = Fluorescence of the enzyme activity in the presence of inhibitor

[0042] The results are shown in Table 3:

Table 3 : Effect of Garcinia extract containing different concentrations of Garcinol and Cambogin on elastase activity

Garcinia Extracts (Percentages of Garcinol and Cambogin)	IC 50
Extract (3% Garcinol + 2% Cambogin)	234 mcg
Extract (20%Garcinol + 10% Cambogin)	140 mcg
Extract (40% Garcinol + 20%Cambogin)	84 mcg
Garcinol 90%	60 mcg
Cambogin 90%	20 mcg

Conclusion : Garcinol is about 3 times as potent as Cambogin in inhibiting elastase activity

[0043] *Example 4 Hyaluronidase inhibitory activity of Benzophenones from Garcinia* The assays were carried out using Sigma method for the enzymatic assay of hyaluronidase. Hyaluronic acid (from human umbilical cord), hyaluronidase (H 3884 from bovine testis), cetyl pyrinidinium chloride and the other reagents were obtained from Sigma. Hyaluronic acid was dissolved in 300mM sodium phosphate buffer pH 5.35. Agarose was dissolved in the same buffer and maintained at 55°C before use. HA solution was preheated to 55° C and mixed with agarose to give a final concentration of 0.5 mg/ml of HA and 0.8% of agarose. Warm HA-agarose mixture (100µl) was dispensed into each well of a microplate and allowed to set. For screening of inhibitors, in another microplate, each well was filled with 100µl of the HA ase (10 units / test in 10 mM sodium phosphate buffer with 77mM sodium chloride pH 7.0) and 100µl of the inhibitor dissolved in DMSO were preincubated at 37 deg C for 10 minutes. A final concentration of 1% DMSO was used which did not have any effect on the enzyme activity. Enzyme activity with DMSO and controls were also kept. After preincubation 100µl from these samples were removed and overlaid onto the pre-set HA/agarose gels wells in triplicates and were incubated at 37 deg C for 45

minutes. After incubation enzyme samples were removed, and each well was filled with 100µl of 10% aqueous cetyl pyridinium chloride. The absorbance was measured in a microplate reader/ Fluostar Optima at 600 nm after 10 minutes in RT. A standard plot was also done with the same procedure using different concentrations of hyaluronic acid and a linearity of R^2 = 0.99 was observed between 0.1 to 0.7 mg/ml of HA concentrations

[0044] The results are expressed as IC 50 values, the concentration at which the compound inhibits half the original hyaluronidase activity. The percentage of inhibition is calculated as follows:

$$\% \text{ Inhibition} = \frac{\{(EC-EA)-(EC-[T-TC]\} \times 100}{(EC-EA)}$$

where EC - Absorbance in the absence of enzyme and inhibitor
EA - Absorbance in the presence of enzyme activity
T - Absorbance of enzyme activity in the presence of inhibitor
TC – Absorbance of the inhibitor alone

[0045] The effects of garcinol and cambogin on hyaluronidase activity are shown in Table 4

Table 4 : Effect of Garcinia extract containing different concentrations of Garcinol and Cambogin on hyaluronidase activity

Garcinia Extracts (Percentages of Garcinol and Cambogin)	IC 50
Extract (3% Garcinol + 2% Cambogin)	102 mcg
Extract (20%Garcinol + 10% Cambogin)	71 mcg
Extract (40% Garcinol + 20%Cambogin)	34 mcg
Garcinol 90%	10 mcg
Cambogin 90%	7 mcg

Conclusion: Garcinol is as potent as Cambogin in inhibiting hyaluronidase activity.

[0046] *Example 5 Trypsin inhibitory activity of Benzophenones from Garcinia*Trypsin inhibitory activity of Benzophenones from Garcinia was assessed by determining the hydrolysis of the synthetic substrate BAPNA (as described by Lottenberg,R, Christensen, U., Jackson, E.M. and Coleman, P.L.(1981) Methods Enzymology 80,341). Aliquots of the enzyme solution (0.25ml of a 1060 units /ml solution in 0.05M Tris buffer P^H 7.8 containing 0.02M $CaCl_2$ and the buffer (or DMSO when the material is insoluble in buffer) with various concentrations of the material and without the material (reference solution) were preincubated at 37 deg C for 10 min. Blank samples were prepared by inactivating the enzyme with 0.5 ml of 2M acetic acid before incubation. The enzymatic reaction was started by adding to the reaction mixture, 1.25ml of 1mM substrate solution (43.5 mg of BAPNA was dissolved in 1ml of DMSO and the solution was brought to 100ml with 0.05M Tris buffer pH 7.8 containing 0.02M $CaCl_2$) The mixture was incubated for 10min and the reaction was stopped by adding 0.5ml of 2M acetic acid. The p-nitroaniline released was measured by using a spectrophotometer at 410nm.

[0047] The percentage inhibition is calculated as follows and Table 5 shows the effects of garcinol and cambogin on trypsin activity.

$$\text{Inhibition ratio (\%)} = \frac{(B-C) - (S-C') \times 100}{(B-C)}$$

where B - absorbance of the reference solution,
C - absorbance of the reference solution blank,
S - absorbance of the test solution and
C' - absorbance of the test solution blank.

Table 5 : Effect of Garcinia extract containing different concentrations of Garcinol Cambogin on trypsin activity

Garcinia Extracts (Percentages of Garcinol and Cambogin)	IC 50
Extract (3% Garcinol + 2% Cambogin)	>1000mcg
Extract (20%Garcinol + 10% Cambogin)	>1000mcg
Extract (40% Garcinol + 20%Cambogin)	415 mcg
Garcinol 90%	300 mcg
Cambogin 90%	180 mcg

Conclusion: Garcinol is about two times as potent as Cambogin in inhibiting trypsin activity.

[0048] The findings presented indicate differential activity in that Cambogin a related benzophenone of Garcinol, is at least two to four times more effective as inhibitor of metalloprotease than Garcinol itself. Additionally, a combination of Garcinol and Cambogin in the extract shows synergistic

activity against elastase, collagenase and hyaluronidase. The lactonization and absence of free hydroxyl group at 7th position in Cambogin is responsible for its enhanced activity against metalloprotease.

[0049] *Example 6 Commercial process for manufacture of Cambogin from Garcinol* Garcinol (50g) obtained as described in prior art was heated at 80-140 degree centigrade in an oven for 1-6 hrs. The material melts and undergoes transformation to get a free flowing powder, Cambogin (yield = 50gms, mp=218-219 ^{o} C)

[0050] *Example 7 Measurement of critical wavelength and UVA/UVB ratio (sunscreen potential)*The critical wavelength λc is a measure of sunscreen"s extinction capacity in UVA range in relation to its overall extinction between 290nm and 400nm. The extinction capacity is given by the area under the extinction curve. λc is calculated as the wavelength at which this area corresponds to 90% of the total area $A_{290-400}$. The higher the critical wavelength of a sunscreen, the better its UVA performance. The critical wavelength is calculated as:

$$\int_{290}^{\lambda c} E(\lambda)d\lambda = 0{,}9 \bullet \int_{290}^{400} E(\lambda)\, d\lambda$$

[0051] The UVA/UVB ratio defines the performance of a sun-

screen in the UVA range (320-400nm) in relation to its performance in the UVB range (290-320nm). It is calculated as the ratio between the area defined by the UVA and UVB extinction capacity. They are calculated as follows:

$$\text{UVA/UVB ratio} = \frac{\int_{320}^{400} A(\lambda)d\lambda \Big/ \int_{320}^{400} d\lambda}{\int_{290}^{320} A(\lambda)d\lambda \Big/ \int_{290}^{320} d\lambda}$$

The findings of the above studies are indicated in Table 6:

Table 6 sunscreen characteristics of Garcinol and Cambogin

	Garcinol 90%	Cambogin 90%
UVA / UVB	0.78	0.16
Critical wavelength	384 nm	334 nm

The findings indicate that Garcinol, is more effective in UVA and cambogin (a related benzophenone of Garcinol) effective in UVB regions.

[0052] *Example 8: Method of analysis for Cyclooxygenase inhibitory activity* The COX (ovine) inhibitor Screening assay directly mea-

sures PGF2α produced in the cyclooxygenase reaction. The prostanoid product is quantified via enzyme immunoassay (EIA) using a broadly specific antibody that binds to all the major prostaglandin compounds. This assay includes both COX-1 and COX-2 enzymes in order to screen isozyme-specific inhibitors. Ovine COX-1 or COX-2 Enzyme is preincubated with & without the inhibitor along with the corresponding controls. Vehicle used is DMSO (1.5%) which did not show any significant effect on enzyme activity. The preincubated mixture is incubated with the substrate for 2 min. 0.1M HCl was added to arrest the reaction & stanous chloride solution was added to stabilize the released prostaglandin"s. Appropriately diluted solution from the above reaction mixture are plated and incubated at room temperature for 18 hrs. followed by colour development with Ellman"s reagent. Note : All the reagents used are from the Cayman chemical COX inhibitory screening assay kit.

[0053] Calculations: Subtract all the readings by B reading. Subtract NSB reading from Bo. Subtract NSB reading from sample reading & divide by 100 to get %B/Bo Plot %B/Bo for Standards S1-S8. Determine the concentration of each sample by identifying the %B/Bo on the Std curve & read-

ing the corresponding values on X-axis. From the difference in pg./ml of PG released in the presence & absence of inhibitor % inhibition is calculated.Where,B (blank) Background absorbance caused by Ellman"s reagent. (NSB Non-specific binding of tracer to the well, Bo (Maximum binding) Maximum amount of tracer that antiserum can bind.)

[0054] Table 7 shows the effects of garcinol and cambogin on COX1 and COX2 activity:

Table 7: Cyclooxygenase Inhibition by Garcinol and Cambogin

Sample	Concentration μg/ml	% Inhibition for COX-1	% Inhibition for COX-2
Garcinol	22	5.0	50
Cambogin	44	50	nil
Diclofenac sodium	17.4	97	98

Conclusions: 1. Garcinol is 10 times more specific inhibitor of Cyclooxygenase-2, which is released during acute inflammatory conditions without affecting the release of beneficial prostaglandin in gastric mucosa . 2. Cambogin is specific inhibitor of Cyclooxygenase-1. 3. Diclofenac sodium is potent inhibitor of both the enzymes. Diclofenac is well documented to be a good anti-inflammatory, however on continued use causes gastric irritation and bleeding, due to non-specific inhibition of cyclooxygenase

[0055] *Example 9: Cytotoxic assay on HEP 2 Cells:* HEP 2 cells were cul-

tured in T-162 flasks with Dulbecco"s Modified Eagle"s Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 0.3% Penicillin (100U/mL)/Streptomycin (100µg/mL). The mixture is maintained under conditions of 10% CO_2 and 90% humidity, at 37 deg C. When confluent, the monolayer of cells was washed twice with calcium and magnesium free phosphate buffered saline (PBS) and trypsinized (0.25% trypsin) at 37 deg C for 1-2 min). Detached cells were transferred into a 50mL centrifuge tube and growth medium added to a volume of 50mL. The suspension was centrifuged at 1500rpm for 5 min., the supernatant discarded and the pellet of cells resuspended in growth medium. Viable cells were counted by trypan blue exclusion using a haemocytometer.

[0056] Cells were plated in 96- well plates for the various experiments. In all cases, the final volume of medium was 200µL per well. Test samples were initially dissolved in DMSO and then diluted to the required concentration in growth medium to 0.5% DMSO, which did not affect the growth of these cells. For each test sample, 8 concentrations were prepared by serial dilution in 0.5% DMSO in growth medium maintaining a constant DMSO concentration. Experiments were carried out in 96 well plates seeded with

5000 cells/well. Cells were plated and initially incubated for 24 hours/ overnight, after which the plating medium was removed and fresh medium containing the extracts, control (1% DMSO in growth medium) or growth medium alone were added. After 3 days incubation, the cell density was determined using the sulforodamine B (SRB) dye method (Skehan et al. (1990)).The SRB assay was carried out as described by Skehan et al., (1990) with modifications). Briefly, at the end of the incubation period, 100µL of ice cold 50% trichloroacetic acid (TCA) was added to each well. Plates were left at °C for 1 hr and then washed five times with tap water to remove TCA, growth medium and dead cells. The plates were allowed to dry, then 50µL of 0.4% w/v SRB stain in 1% acetic acid in distilled water, was added to each well and left in contact with the cells for 30 minutes at room temperature. at the end of the staining period, the plates were washed 5-6 times with 1% aqueous acetic acid to remove any unbound dye, dried and then stored at room temperature if necessary. On the day of reading the plates, 100µL of 10mM aqueous Tri base (Tris[hydroxymethyl]aminomethane) was added to each well to solubilize the dye. The plates were shaken for 5 minutes and absorbance readings taken at 538nm using

a Fluostar optima(BMG) microplate Reader Note: All the reagents used were from Sigma. The percentage growth of cells as compared to the control (0.5% DMSO) was calculated. IC_{50} values were obtained from graphs of percentage growth vs concentration. Mean optical density (OD ±SD as a percent of control) was calculated for each concentration from the six replicate wells in a single plate. This data was used to plot a dose response curve from which IC_{50} values were obtained. The mean IC_{50} values ±SD for compounds are provided in Table 8:

Table 8: IC50 values for garcinol and cambogin in cytotoxicity assay

Compound	IC50 value (µg/ml)
Garcinol	75 ± 3.4
Cambogin	3.1± 0.4

[0057] Cosmetic Formulations based on Policosanol are described in the following examples

[0058] *Example 10 Sebum Control, blemish free, anti-acne gel*

Part A	
Polysaccharides from tamarind	2.0%
Tetrahydrocurcumin	0.01%
Garcinol	0.1%
THP (Tetrahydropiperine)	0.01%
Part B	
Deionized water	97.4%

Heat Part B to 60 degree centigrade, and add part A to Part B under stirring and cool to the desired fill temperature.

(THP and tetrahydrocurcumin are ingredients from Sabinsa Corp, Piscataway, NJ USA derived from black pepper fruit and Turmeric respectively, which function as topical permeation enhancer and skin tone lightener respectively)

[0059] *Example 11 Skin Rejuvenating Lotion*

Ingredients	% (w/w)
Phase A	
Polyglyceryl-3 methyl glucose distearate	3.5
Glyceryl monostearate	2.50
Dicapryl ether	5.0
Caprylic/caproic esters	5.0
Propylene glycol Dicaprylate/dicaprate	3.5
Coriander oil	2.0
Cetyl alcohol	1.5
Tetrahydrocurcumin	0.1
Cambogin	0.2
Phase B	
Glycerin	3.0
Propylene glycol	3.0
Blepharis edulis extract	1.0
Rosemary extract	0.5
Methylparaben	0.15
Deionized water	QS

Combine A, stir and heat to 65 degree centigrade. Combine B, stir and heat to 65 degree centigrade. Add A to B under stirring. Homogenize avoiding foaming, cool to fill temperature

[0060] *Example 12 Sunscreen and Anti-wrinkle Lotion*

Ingredients	% (w/w)
Phase A	
Polyglyceryl-3 methyl glucose distearate	3.5
Glyceryl monostearate	2.50
Dicapryl ether	5.0
Caprylic/caproic esters	5.0
Propylene glycol Dicaprylate/dicaprate	3.5
Octyl methoxycinnamate	7.5
Benzophenone 4	1.5
Cetyl alcohol	1.5
Garcinol	0.2%
Cambogin	0.2%
Phase B	
Glycerin	3.0
Propylene glycol	3.0
Methylparaben	0.15
Deionized water	QS

Procedure :Combine A, stir and heat to 65 degree centigrade. Combine B, stir and heat to 65 degree centigrade. Add A to B under stirring. Homogenize avoiding foaming, cool to fill temperature.

[0061] *Example 13 Anti-dandruff, anti-seborrhic, Hair Conditioning Liquid*

Ingredients	% Concentrations
Garcinol	0.2
Policosanol	0.5
Silicone oil	10%
Lauriforte (Sabinsa Corp)	50%
Flax seed oil	22%
Light Liquid Paraffin	QS

Procedure : Heat the oil to about 60 degrees, add each of the ingredients , stir until dissolution, cool to the fill temperature

Lauriforte™ is coconut oil fraction rich in medium-chain triglycerides